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DNA Adducts As Biomarkers for Assessing Exposure to Polycyclic Aromatic Hydrocarbons in Tissues from Xuan Wei Women with High Exposure to Coal Combustion Emissions and High Lung Cancer Mortality

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The high lung cancer rate in Xuan Wei, China, is associated with smoky coal use in unvented homes, but not with wood or smokeless coal use. Smoky coal combustion emits higher polycyclic aromatic hydrocarbon (PAH) concentrations than wood combustion. This study used DNA adducts as biomarkers for human exposure to PAH from combustion emissions. DNA adducts were determined by enzyme-linked immunosorbent assays (ELISA) in placentas and peripheral and cord white blood cells (WBC) from Xuan Wei women burning smoky coal or wood and from Beijing women using natural gas. Color ELISA gave positive results in 58, 47, and 5% of the placentas from Xuan Wei women burning smoky coal without and with chimneys, and from Beijing women, respectively. Fluorescence ELISA indicated that 46, 65, 56, and 25% of placentas were positive from Xuan Wei women who lived in houses without and with chimneys, Xuan Wei women burning wood, and Beijing controls, respectively. Peripheral WBC samples were positive in 7/9, 8/9, and 3/9 for the Xuan Wei women who lived in houses without and with chimneys and Beijing women, respectively. PAH–DNA adducts were detected in a higher percentage of placentas from Xuan Wei women living in houses exposed to smoky coal or wood emissions than from those of the Beijing controls. No dose–response relationship was observed between the air benzo[*a*]pyrene concentrations and DNA adduct levels or percentage of detectable samples. The results suggest that DNA adducts can be used as a qualitative biomarker to assess human exposure to combustion emissions.

Introduction

Rural Xuan Wei County in Yunnan Province, China, has a population of about 1 million, and most of them (90%) are farmers. The lung cancer mortality rate in this county is unusually high. The annual age-adjusted (to China population in 1964) lung cancer mortality rate in Xuan Wei from 1973 to 1979 was 27.7/100,000 in males, among China's highest, and 25.3/100,000 in females, China's highest (*1*) (Table 1). Three Xuan Wei communes with the highest lung cancer mortality rates have rates as high as 118.0/100,000 in males and 125.6/100,000 in females. These lung cancer mortality rates are much higher than the national rate for China or the rate for Beijing. The high lung cancer rate cannot be attributed to occupational exposure or tobacco smoke, especially in females who are mostly nonsmokers (smoking rate < 0.1%). The similarity of lung cancer mortality rates between males (smoking rate > 40%) and females is unusual. Traditionally, Xuan Wei residents have used one of three fuels,

Table 1. Annual lung cancer mortality rates in Xuan Wei.

Location	Time period	Mortality rate per 100,000[a] Males	Females	Combined
Xuan Wei County	1973–79	27.7	25.3	
Cheng Guan, a smoky-coal-burning (100%) commune in Xuan Wei	1973–79	118.0	125.6	
Re Shui, a wood- (67%) and smokeless-coal-burning commune in Xuan Wei	1973–79			2.1
Yunnan Province	1973–75	4.3	1.5	
Beijing	1973–75	12.0	8.6	
China	1973–75	6.8	3.2	
United States	1970	30.0	6.3	

[a]Age-adjusted to 1964 China population.

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Table 2. Polycyclic aromatic hydrocarbon analysis of the organic extracts of indoor air particles (PM_{10}) from burning different fuels during cooking in Xuan Wei homes.

	Smoky coal		Smokeless coal		Wood	
PAH[a]	μg/g Organics	μg/m³ Air	μg/g Organics	μg/m³ Air	μg/g Organics	μg/m³ Air
Benz[*a*]anthracene	1450	32.83	2060	1.01	264	4.20
5-Methylchrysene	729	16.51	464	0.23	2	0.03
Benzofluoranthenes	1370	31.02	3780	1.86	194	3.08
Benzo[*a*]pyrene	850	19.25	1160	0.57	204	3.24
Indeno[1,2,3-*cd*]pyrene	529	11.98	915	0.45	127	2.02
Dibenzo[*ae*]pyrene	771	17.46	1310	0.64	BDL	BDL
Dibenz[*aj*]acridine	31	0.70	14	0.007	BDL	BDL

Abbreviations: PAH, polycyclic aromatic hydrocarbon; BDL, below detection limit.

[a]Compounds presented are those classified as "sufficient evidence of carcinogenicity in experimental animals" (*19*).

smoky coal, smokeless coal, or wood, for heating and cooking in unvented homes. The lung cancer mortality rate is highly correlated with domestic use of smoky coal, in contrast to wood and smokeless coal use (*1*). Previous indoor air characterization studies showed that indoor air samples collected in Xuan Wei homes during cooking with smoky coal contain mostly (51%) submicron particles with about 80% organic content, including high concentrations (43% of the organic mass) of polycyclic aromatic hydrocarbons (PAHs) (*1*). [See Table 2 for PAH analysis of the organic extracts of indoor air particles from burning the three fuels (*2*)]. The organic extract of the indoor air particles from smoky coal combustion was mutagenic in the Ames Salmonella mutagenicity assay and the L5178Y $TK^{+/-}$ mouse lymphoma cell mutation assay and was a potent tumor initiator and a complete carcinogen in Sencar mouse skin assays (*2*). The PAH and polar fractions of the organic extracts were the most mutagenic. In comparison with smoky coal, air particle samples from homes in the wood-burning commune, which has a low rate of lung cancer, mostly consisted of larger particles of lower PAH concentrations and had lower mutagenicity and tumorigenicity (*1,2*). Combustion of smokeless coal generates a lower level of particles than does combustion of smoky coal, and these particles contain fewer (30%) organics.

Formation of DNA adducts by covalent binding to chemicals with electrophilic properties is considered the initiating event leading to mutation and/or neoplastic transformation. Based on this rationale, DNA adduct formation has been used as a dosimeter to assess human exposure to environmental carcinogens and also to assess genotoxic effects resulting from exposure. Methods for detecting carcinogen–DNA adducts in exposed individuals include immunoassays (*3*), synchronous fluorescence spectrophotometry (*4*), mass spectroscopy (*5*), and ^{32}P-postlabeling (*6*). Enzyme-linked immunosorbent assays (ELISA), using antibodies that recognize DNA modified by benzo[*a*]pyrene (BaP) diol epoxide-I (BPDE-I) have been used to detect DNA adducts in tobacco smokers, coke oven workers, and foundry workers (*3,7–10*). These antibodies also recognize structurally related diol epoxide adducts and provide a general measurement of PAH diol epoxide–DNA adduct levels in human populations. The objectives of this study were to use DNA adduct levels as a biomarker to assess human exposure to PAH from coal and wood combustion emissions in Xuan Wei residents and explore the possible link between DNA adduct formation and lung cancer mortality rate in Xuan Wei. The tissues analyzed were placenta, cord blood, and peripheral blood from Xuan Wei women exposed to wood and smoky coal emissions and from Beijing women serving as controls.

Materials and Methods

Human Tissues

Peripheral blood, cord blood, and placentas from term, normal pregnancies were collected from Xuan Wei women who use either smoky coal (with and without chimneys) or wood and from Beijing women who use natural gas at home. No peripheral or cord blood samples were collected for the wood-using women due to logistic difficulties. All the women were nonsmokers and were similar in age: 25 ± 3, 26 ± 3, 25 ± 5, and 27 ± 3 (mean ± SD) for the Xuan Wei women using smoky coal without chimneys, Xuan Wei women using smoky coal with chimneys, Xuan Wei women using wood, and Beijing controls, respectively.

Placental Tissues. Placental tissues (2–3 g) were homogenized (10%, w/v) in Tris buffer (10 mM Tris, 1 mM EDTA, 0.4 M NaCl, pH 7.9). After addition of sodium dodecyl sulfate (0.2%) and proteinase K (200 μg/mL), the samples were incubated for 2 hr at 37°C then shaken for 10 min with an equal amount of buffer-saturated phenol. The aqueous layer was separated, and the nucleic acids were precipitated with cold ethanol at -20°C. The crude DNA was dried and transported to the United States for further purification by treatment with RNAse and proteinase K and extraction with chloroform:isoamyl alcohol:phenol and chloroform:isoamyl alcohol as described previously (*11*).

Peripheral and Cord Blood. The blood samples (20–50 mL) were collected in heparinized plastic tubes. The buffy coat containing mostly white blood cells (WBC) was separated from red blood cells by centrifugation. The buffy coat was further purified by lysing the residual red blood cells with 0.32 M sucrose, 10 mM Tris, 5 mM $MgCl_2$, 1% Triton X100, pH 7.6. Buffy coat cells were separated and DNA was isolated by the same procedures used for the placental DNA.

ELISA for BPDE-I–DNA Antigenicity

Placental and blood samples were assayed by competitive fluorescence and color ELISA. The fluorescence ELISA was carried out essentially as described previously in 96-microwell plates coated with 0.2 ng BPDE-I–DNA (5 adducts/10^3 nucleotides or 15 pmole/μg) (*3*). Rabbit polyclonal antibody no. 29 was used at a 1:1,600,000 dilution. Goat anti-rabbit IgG-alkaline phosphatase (Sigma Chemical Co., St Louis, MO) was used at a 1:400 dilution, and the substrate was 4-methylumbelliferyl phosphate. For the color ELISA, all steps were the same as in the fluorescence ELISA except that plates were coated with 5 ng BPDE-I–DNA/well, antibody was diluted 1:30,000, and the substrate was *p*-nitrophenylphosphate. Absorbance was measured at 405 nm. Samples with greater than 20% inhibition were considered detectable, and samples showing less than 20% inhibition were classified as nondetectable. The limit of detection was estimated to be about 0.03 fmole/μg DNA for the fluorescence assay and 0.08 fmole/μg DNA for the color assay. For the fluorescence assay, results are given as the mean of a single assay with triplicate wells. In the color assay, placental

samples were tested in duplicate wells in two separate assays, whereas blood samples were tested in duplicate wells in a single assay.

Personal Exposure and Home Concentration for BaP

Personal air sampling was conducted to assess the BaP exposure on most of the Xuan Wei women who used smoky coal and donated their tissues for the study. Fourteen of the Xuan Wei women in homes without chimneys and sixteen of the Xuan Wei women in homes with chimneys carried active personal samplers for monitoring air particles at a flow rate of 2 L/min for 24 hr. The total particulate mass and mean 24-hr particulate concentrations were determined. BaP concentrations were estimated based on the calculation that the concentration of BaP is equal to about 0.0576% of the indoor air particulate concentration, according to a Xuan Wei home monitoring study described below.

In a separate study, air monitoring was conducted in five Xuan Wei homes using smoky coal without chimneys and in two homes burning wood without chimneys. Indoor air particles ($< 10\ \mu m$) were collected on a glass-fiber filter by a particulate sampler with a flow rate of 4 L/min for 24 hr and were monitored for 1 week in these homes. The indoor air particulate concentrations in these homes were determined. The organic extracts of these particulate samples were analyzed for quantitative PAH concentrations, including BaP concentrations, by GC–MS (*12*). Because no personal or home air monitoring was conducted in the Beijing subjects, the outdoor concentration in Beijing reported previously (*13*) was used to estimate the indoor BaP exposure of the Beijing subjects. This estimate assumes that PAH emissions from natural gas combustion contribute insignificant amounts of PAH to indoor air and the major sources of indoor PAH were from outdoor sources through infiltration.

Results

The results of personal air sampling showed that the Xuan Wei women burning smoky coal in homes without chimneys were exposed to a BaP concentration twice that of the Xuan Wei women burning smoky coal in homes with chimneys (Table 3). Indoor air monitoring data showed that the Xuan Wei women burning smoky coal without chimneys were exposed to five times the BaP concentrations as the Xuan Wei women burning wood in their homes. In comparison with the Beijing controls, it is expected that the BaP concentrations in Xuan Wei homes burning coal or wood would be higher than the Beijing homes burning natural gas.

Greater than 50% of the total Xuan Wei women using smoky coal showed detectable BaP-related DNA adducts in placental samples in both fluorescence and color ELISA (Table 3). In the color assay, a higher percentage (58%) of the placentas from the Xuan Wei women burning smoky coal without chimneys had detectable adducts than in the placentas from the Xuan Wei women burning smoky coal with chimneys (47%). In the fluorescence assay, the Xuan Wei women who lived in homes without chimneys did not show a higher percentage of placental samples with detectable adducts than the women living in houses with chimneys. Most (56%) of the women burning wood in homes also showed detectable placental DNA adducts. A relatively low percentage of the Beijing women's placentas were found to have detectable DNA adducts (i.e., 25% in the fluorescence assay and 5% in the color assay). DNA adduct levels of the positive placental samples are similar in the three groups of Xuan Wei women, ranging from 3.0×10^{-8} to 4.0×10^{-8} in the fluorescence assay and 10.2×10^{-8} to 10.5×10^{-8} in the color assay. Among the Xuan Wei women using smoky coal or wood, no dose–response relationship was found between BaP air concentrations and BaP-related DNA adduct formation.

Table 4 shows the results of the ELISA for cord and peripheral WBC samples. This study is limited by the small number of blood samples available. However, the data from the fluorescence ELISA did show a trend with more detectable DNA samples in peripheral blood samples from Xuan Wei women using smoky coal than in the Beijing samples. Most blood samples had nondetectable levels of adducts in the color assay. The color ELISA has been shown to be less sensitive than the fluorescence assay (*14*).

Discussion

Xuan Wei residents are exposed to complex mixtures of coal or wood combustion emissions containing carcinogenic PAH.

Table 3. BPDE-I–DNA antigenicity of placental samples by competitive ELISA.[a]

Exposure	Lung cancer mortality per 100,000[b]	Personal BaP exposure, ng/m³	Home BaP concentration, ng/m³	Fluorescence assay % Detected[c]	Fluorescence assay fmole/μg DNA[d] (adducts/10⁸)	Color assay % Detected[c]	Color assay fmole/μg DNA[d] (adducts/10⁸)
Smoky coal							
No chimney	174	383 ± 225	2283 ± 1904	46(6/13)	0.12 ± 0.13 (4.0 ± 4.3)	58(11/19)	0.32 ± 0.08 (10.5 ± 2.6)
With chimney		184 ± 136		65(11/17)	0.09 ± 0.05 (3.0 ± 1.7)	47(9/19)	0.31 ± 0.09 (10.2 ± 3.0)
Total				57(17/30)	0.10 ± 0.08 (3.3 ± 2.6)	52(20/38)	0.31 ± 0.08 (10.3 ± 2.6)
Wood	2.1		481	56(9/16)	0.11 ± 0.06 (3.6 ± 2.0)		
Natural gas (Beijing control)	10.3		17	25(4/16)	0.28 ± 0.16 (9.2 ± 5.3)	5.3(1/19)	0.23 (7.6)

Abbreviations: BPDE-I, benzo[*a*]pyrene diol epoxide-I; BaP, benzo[*a*]pyrene; ELISA, enzyme-linked immunosorbent assay.

[a]Data shown in this table are means ± SD where applicable.

[b]Age-adjusted to 1964 China population.

[c]Parentheses show the number of samples detected/number of total samples assayed.

[d]Means ± SD for samples with detectable adducts.

Table 4. BPDE-I–DNA antigenicity of white blood cell samples by competitive ELISA.

Exposure	Fluorescence assay		Color assay	
	No. detected/no. assayed	fmole/μg DNA[a] (adducts/10^8)	No. detected/no. assayed	fmole/μg DNA[a] (adducts/10^8)
Smoky coal				
Peripheral blood				
No chimney	7/9	0.24 ± 0.05 (7.9 ± 1.7)	3/11	0.51 ± 0.04 (16.8 ± 1.3)
With chimney	8/9	0.23 ± 0.12 (7.6 ± 4.0)	1/11	0.39 (12.9)
Cord blood				
No chimney	3/8	0.25 ± 0.13 (8.3 ± 4.3)	0/11	—
With chimney	7/7	0.22 ± 0.06 (7.3 ± 2.0)	1/10	0.27 (8.1)
Natural gas (Beijing control)				
Peripheral blood	3/9	0.12 ± 0.07 (4.0 ± 2.3)	1/8	0.39 (12.8)
Cord blood	3/6	0.19 ± 0.06 (6.3 ± 2.0)	0/7	—

Abbreviations: BPDE-I, benzo[*a*]pyrene diol epoxide-I; ELISA, enzyme-linked immunosorbent assay.
[a]Mean ± SD for samples with detectable adducts.

This study investigated *a*) the relationship between air concentrations of BaP and the extent of formation of DNA adducts by BPDE-I and related PAH-diol epoxides, measured by ELISA, in humans exposed to combustion emissions and *b*) the possible linkage of DNA adduct formation to human lung cancer mortality in Xuan Wei.

Human placental tissues are more readily available than other human tissues for human dosimetry studies. This study investigated PAH–DNA adducts in placentas and blood to determine if placental tissues are appropriate for human monitoring. Using immunoaffinity chromatography with anti-BPDE-I–DNA antibodies, HPLC–synchronous fluorescence spectroscopy and GC–MS, Manchester et al. (*15*) have confirmed the presence of BPDE-I–DNA adducts (at 1 adduct/10^7 nucleotides) in 10 of 28 placenta DNA samples isolated from both smokers (15 subjects) and nonsmokers (13 subjects). The presence of this adduct was not related to smoking. Everson et al. (*7*) showed a small but not statistically significant increase in adduct levels between smokers' (1.8×10^{-7}) and nonsmokers' (1.2×10^{-7}) placental DNA adduct levels by ELISA. The levels of PAH–DNA adducts as reported in the Xuan Wei women are comparable or slightly lower than the levels found in these two placental studies. If air is the major source of PAH exposure, one would expect to detect higher levels of PAH–DNA adducts in Xuan Wei women exposed to smoky coal emissions than in nonsmoking women in the United States.

In this study, PAH–DNA was detected in 56% (9/16) of the placental samples from Xuan Wei women burning wood. Using the ^{32}P-postlabeling assay, Reddy et al. (*16*) reported a lack of BaP-related DNA adducts or other residential wood combustion-related adducts in placentas or WBC of women. The authors acknowledged that the BaP exposure of these women was not monitored and it was difficult to link the results of DNA adducts to the exposure to wood combustion emissions. Our previous studies showed that wood combustion emits PAH compounds, including BaP, although the PAH concentrations are not as high as the emissions from coal combustion (see Table 2). Emissions from wood combustion contained high concentrations of polar compounds, such as substituted PAH with functional groups (e.g., phenols, oxygenated PAH) (*1*). In this study, the percentage of wood-burning women showing detectable PAH–DNA and their mean adduct levels were similar to the Xuan Wei women burning smoky coal. This may be due to the detection of BaP-related adducts and adducts of polar PAH compounds that are present in the wood combustion. Little information is available on the DNA adduct formation of these polar compounds. Further investigations are needed to determine if the polyclonal antibody used in this study cross-reacts with DNA adducts of the polar PAH.

Coke oven workers are exposed to the emissions from coal pyrolysis, containing high concentrations of PAH, similar to emissions from smoky coal combustion. In this study we found detectable BaP-related adducts in 57 and 52% of the placental samples from the Xuan Wei women burning smoky coal in the fluorescence and color assays, respectively. Most of the Xuan Wei peripheral WBC samples showed detectable DNA adducts. Haugen et al. (*4*) reported that coke oven workers exposed to BaP concentrations of 1 to 9 μg/m^3 had detectable PAH–DNA adducts in 34% of the workers' lymphocytes by ultrasensitive enzymatic radioimmunoassay (USERIA). The mean adduct level, 1.3×10^{-7}, is higher than the adduct levels in the Xuan Wei peripheral WBC samples in this study. The lower PAH exposure of the Xuan Wei women and the difference in sensitivity of the USERIA and ELISA may be the reason for the lower DNA-adduct levels reported here. Harris et al. (*17*) reported that 64% (18/27) of coke oven workers had detectable adducts by USERIA, ranging from 3.6 to 14.5×10^{-7}. Van Schooten et al. (*8*) reported that 47% (24/51) of the coke oven workers' and 30% of controls' WBC samples were positive by ELISA, with mean adduct levels of 5.1×10^{-8} and 2.7×10^{-8}, respectively. The difference between the coke oven workers and controls was not statistically significant. Smokers had significantly higher levels of PAH–DNA adduct levels. There was no significant correlation between PAH–DNA adducts in blood and concentrations of PAH in air and 1-hydroxypyrene in urine.

In our study, a higher percentage of placental DNA from Xuan Wei women exposed to wood and smoky coal emissions showed detectable PAH–DNA adducts than Beijing controls. In Xuan Wei women, no dose–response relationships between BaP exposure and placental DNA adduct levels or percentage of samples with detectable DNA adducts were found. This may be due to the confounding factor of dietary intake of PAH. Although inhalation is an important route of exposure, Buckley et al. (*18*) showed that diet is a significant route of exposure to PAH. In the United States, PAH exposure from inhalation, in general, is less than that from ingestion. The variation in dietary intake of PAH and the differences in persistence and repair of DNA adducts among individuals may be responsible for the lack of a dose–response relationship between air exposure of PAH and DNA adduct formation in this study.

This study also showed no relationship between percentage of the samples detected with DNA adducts and lung cancer mortality rate. The Xuan Wei women from smoky-coal communes with high lung cancer mortality did not show higher levels or a higher percentage of detectable PAH–DNA than the Xuan Wei women from a wood-burning commune with a low lung cancer mortality rate. These results suggest that the PAH–DNA adducts, measured by ELISA, can be used as a qualitative biomarker of exposure to combustion emissions but not as a predictor for cancer risk.

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REFERENCES

1. Mumford, J. L., He, X. Z., Chapman, R. S., Cao, S. R., Harris, D. B., Li, X. M., Xian, Y. L., Jiang, W.Z., Xu, C. W., Chuang, J. C., Wilson, W. E.,and Cooke, M. Lung cancer and indoor air pollution in Xuan Wei, China. Science 235: 217–220 (1987).
2. Mumford, J. L., Helmes, C. T., Lee, X., Seidenberg, J., and Nesnow S. Mouse skin tumorigenicity studies of indoor coal and wood combustion emissions from homes of residents in Xuan Wei, China with high lung cancer mortality. Carcinogenesis 11: 397–403 (1990).
3. Perera, F. P., Hemminki, K., Young, T. L., Brenner, D., Kelly, G., and Santella, R. M. Detection of polycyclic aromatic hydrocarbon-DNA adducts in white blood cells of foundry workers. Cancer Res. 48: 2288–2291 (1988).
4. Haugen, A., Becher, G,, Benestad, C., Vahakangas, K., Trivers, G. E., Newman, M. J., and Harris, C. C. Determination of polycyclic aromatic hydrocarbons in the urine, benzo[*a*]pyrene diol epoxide-DNA adducts in lymphocyte DNA, and antibodies to the adducts in sera from coke oven workers exposed to measured amounts of polycyclic aromatic hydrocarbons in the work atmosphere. Cancer Res. 46: 4178–4183 (1986).
5. Weston, A., Rowe, M. L., Manchester, D. K., Farmer, P. B., Mann, D. L., and Harris, C. C. Fluorescence and mass spectral evidence for the formation of benzo(a)pyrene antidiol epoxide-DNA and hemoglobin adducts in humans. Carcinogenesis 10: 251–257 (1989).
6. Randerath, E., Miller, R. H., Mittal, D., Avitts, T. A., Dunsford, H. A., and Randerath, K. Covalent DNA damage in tissues of cigarette smokers as determined by ^{32}P-postlabeling assay. J. Natl. Cancer Inst. 81: 341–347 (1989).
7. Everson, R. B., Randerath, E., Santella, R. M., Cefalo, R. C., Avitts, T. A., and Randerath, K. Detection of smoking-related covalent DNA adducts in human placenta. Science 231: 54–57 (1986).
8. van Schooten, F. J., van Leeuwen, F. E., Hillebrand, M. J. X., de Rijke, M. E., Hart, A. A. M., van Veen, H. G., Oosterink, S., and Kriek, E. Determination of benzo[*a*]pyrene diol epoxide-DNA adducts in white blood cell DNA from coke-oven workers: the impact of smoking. J. Natl. Cancer Inst. 82: 927–933 (1990).
9. Poirier, M. C., Santella, R., Weinstein, I. B., Grunberger, D., and Yuspa, S. H. Quantitation of benzo[*a*]pyrene-deoxyguanosine adducts by radioimmunoassay. Cancer Res. 40: 412–416 (1980).
10. Santella, R. M., Lin, C. D., Cleveland, W. L., and Weinstein, I. B. Monoclonal antibodies to DNA modified by a benzo[*a*]pyrene diol epoxide. Carcinogenesis 5: 373–377 (1984).
11. Gupta, R. C. Nonrandom binding of the carcinogen N-hydroxy-2-acetylaminofluorene to repetitive sequences in rat liver DNA *in vivo*. Proc. Natl. Acad. Sci. U.S.A. 81: 6943–6947 (1984).
12. Petersen, B. A., and Chuang, J. C. Methodology of fractionation and partition of diesel exhaust particulate samples. In: Toxicological Effects of Emissions from Diesel Engines (J. Lewtas, Ed.), Elsevier, New York, 1982, pp. 51–57.
13. Daisey, J. M., Kneip, T. J., Wang, M.-X., Ren, L.-X., and Lu, W.-X. Organic and elemental composition of airborne particulate matter in Beijing, Spring 1981. Aerosol Sci. Technol. 2: 407–415 (1983).
14. Santella, R. M., Weston, A., Perera, F. P., Trivers, G. T., Harris, C. C., Young, T. L., Nguyen, D., Lee, B. M., and Poirier, M. C. Interlaboratory comparison of antisera and immunoassays for benzo[a]pyrene-diol-epoxide-I-modified DNA. Carcinogenesis 9: 1265–1269 (1988).
15. Manchester, D. K., Weston, A., Choi, J.-S., Trivers, G. E., Fennessey, P. V., Quintana, E., Farmer, P. B., Mann, D. L., and Harris, C. C. Detection of benzo[a]pyrene diol epoxide-DNA adducts in human placenta. Proc. Natl. Acad. Sci. U.S.A. 85: 9243–9247 (1988).
16. Reddy, M. V., Kenny, P. C., and Randerath, K. ^{32}P-Assay of DNA adducts in white blood cells and placentas of pregnant women: lack of residential wood combustion-related adducts but presence of tissue-specific endogenous adducts. Teratog. Carcinog. Mutagen. 10: 373–384 (1990).
17. Harris, C. C., Vahakangas, K., Newman, M. J., Trivers, G. E., Shamsuddin, A., Sinopoli, N., Mann, D. L., and Wright, W. E. Detection of benzo[a]pyrene diol epoxide-DNA adducts in peripheral blood lymphocytes and antibodies to the adducts in serum from coke oven workers. Proc. Natl. Acad. Sci. U.S.A. 82: 6672–6676 (1985).
18. Buckley, T. J., and Lioy, P. J. An examination of the time-course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. Br. J. Ind. Med. 49: 113–124 (1992).
19. IARC. Polynuclear Aromatic Compounds, Part 1. Chemical, Environmental and Experimental Data. In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 32. International Agency for Research on Cancer, Lyon, 1983, pp. 95–445.